

The diagram illustrates the three-step PCR process for generating a pool of mutant *argA* genes. It shows the progression from a wild-type *argA* gene to a pool of mutant genes through three successive PCR steps, each introducing a mutation. The final product is a pool of mutant *argA* genes, which are then digested with *Nco*I and *Hind*III for further analysis.

First PCR step: The wild-type *argA* gene (wt) is amplified using primers P1 and P2. The resulting PCR product contains a mutation (indicated by a wavy line) introduced by primer P1.

Second PCR step: The PCR product from the first step is amplified using primers P2 and P3. The resulting PCR product contains a second mutation (indicated by a wavy line) introduced by primer P3.

Third PCR step: The PCR product from the second step is amplified using primers P2 and P3. The resulting PCR product contains a third mutation (indicated by a wavy line) introduced by primer P3.

The final product is a **Pool of mutant *argA* genes**, which are then digested with *Nco*I and *Hind*III for further analysis.